1,114,155

PATENT SPECIFICATION

NO DRAWINGS

1,114,155

Inventors: GEORGE GERALD BADCOCK and WILFRED JAMES CECIL DYKE

Date of filing Complete Specification: 20, Aug., 1965.

Application Date: 24 Aug., 1964. No. 34538/64.

Complete Specification Published: 15 May, 1968.

Index at acceptance:—C2 C(2A1, 2A5, 2A8, 2A9, 2A12, 2A14, 2R20, 2S21, 2S22); **A5 B**(1H, 2H, 7); **A5 E**(1A3B3, 1C3B3)

Int. Cl.:—C 07 c 129/12

COMPLETE SPECIFICATION

Guanidino Derivatives

R¹ R²

ERRATA

SPECIFICATION No. 1,114,155

Page 3, line 6, *for* "by weight" *read* "by weight"

Page 3, line 49, *after* "of" *insert* "a" (first occurrence)

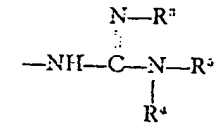
Page 6, Table VI, Example 5, *under* "Fusarium nivale" *delete* "43%"

Page 6, Table VI, Example 5, *under* "Phoma betae" *insert* "43%"

THE PATENT OFFICE
18th June 1968

vity to *pseudomonas pyocyanea*, *aureus* and/or *escherischia coli*. Furthermore, activity has been shown by a number of the new compounds against plant pathogenic fungi including *inter alia Erysiphe graminis*, *Venturia inaequalis*, *Podisphaera leucotricha*, *Uromyces fabae*, *Botrytis fabae*, and *Cercospora melonis*. In addition to the antifungal and antibacterial effects in humans, antiviral, analgesic and antiheparin activities have been demonstrated by some of the compounds. The new compounds are desirably utilised as their acid addition salts and in general are water soluble, this being in contrast to previously proposed antifungal compounds such as griseofulvin. The compounds further have a low toxicity. They also have the important practical advantage that they can be prepared from readily available starting materials, as is also described below.

According to the present invention we provide compounds of the general formula



(II)

where R^2, R^1 and R^3, which may be the same or different, are hydrogen atoms or alkyl groups having 1—4 carbon atoms.

While the alkylene chains R^6, R^7 and R^5 may each contain, for example, up to 20 carbon atoms, they preferably contain not more than 15 carbon atoms and advantageously not more than 12 carbon atoms. In compounds which are particularly useful against *Candida albicans* the preferred chain length for these alkylene groups is of the order of 6 carbon atoms. In compounds which are particularly

[*Price*

SEE ERRATA SLIP ATTACHED

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COMPLETE SPECIFICATION

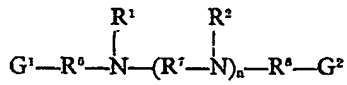
Guanidino Derivatives

We, EVANS MEDICAL LIMITED, a British Company of Speke Boulevard, Speke, Liverpool, Lancashire, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention is concerned with novel guanidino derivatives.

We have found that certain guanidino derivatives, which are described in detail below, possess valuable activity against human and/or animal and/or plant pathogens. The new compounds possess antifungal activity particularly against the pathogenic fungus *Candida albicans*, which gives rise to a variety of diseases in humans, and antibacterial activity against, for example, such organisms as *pseudomonas pyocyaneus, staphylococcus aureus* and/or *escherischia coli*. Furthermore, activity has been shown by a number of the new compounds against plant pathogenic fungi including *inter alia Erysiphe graminis, Venturia inaequalis, Podisophoera leuchroricha, Uromyces fabae, Botrytis fabae,* and *Cercospora melonis*. In addition to the antifungal and antibacterial effects in humans, antiviral, analgesic and antiheparin activities have been demonstrated by some of the compounds. The new compounds are desirably utilised as their acid addition salts and in general are water soluble, this being in contrast to previously proposed antifungal compounds such as griseofulvin. The compounds further have a low toxicity. They also have the important practical advantage that they can be prepared from readily available starting materials, as is also described below.

According to the present invention we provide compounds of the general formula



(I)

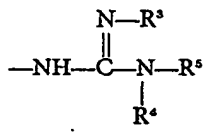
and their physiologically acceptable acid addition salts, where

R^1 and each radical R^2, which may be the same or different, are hydrogen atoms or alkyl groups,

R^6, and each group R^7 and R^8, which may be the same or different, are straight or branched alkylene groups separating adjacent nitrogen atoms by chains of at least two carbon atoms, the total number of carbon and nitrogen atoms in the straight chain between the two groups G^1 and G^2, excluding branching groups, being always greater than 12,

n is an integer from 0—4, and

G^1 and G^2, which may be the same or different, have the formula



(II)

where R^3, R^4 and R^5, which may be the same or different, are hydrogen atoms or alkyl groups having 1—4 carbon atoms.

While the alkylene chains R^6, R^7 and R^8 may each contain, for example, up to 20 carbon atoms, they preferably contain not more than 15 carbon atoms and advantageously not more than 12 carbon atoms. In compounds which are particularly useful against *Candida albicans* the preferred chain length for these alkylene groups is of the order of 6 carbon atoms. In compounds which are particularly

[*Price*

SEE ERRATA SLIP ATTACHED

useful against bacteria infecting humans and against plant pathogenic fungi, the preferred chain lengths for these alkylene groups is greater than 6 carbon atoms, for example 8, 10 or 12 carbon atoms.

The preferred compounds are those in which R^1 and R^2 are each a methyl group or a hydrogen atom.

Particularly preferred compounds according to the invention are N,N'-bis-(3-guanidinopropyl)hexamethylenediamine, N,N' - bis-(6 - guanidinohexyl)hexamethylenediamine, N,N' - bis - (6 - guanidinohexyl)trimethylenediamine and bis - (8 - guanidinooctyl)-amine which in our tests have shown marked activity against *Candida albicans*, and bis-(10-guanidinodecyl)amine and bis-(12-guanidinododecyl)amine which have shown marked activity against certain plant pathogenic fungi.

The last named compound has also shown particularly useful antibacterial activity and bis - (8 - guanidinooctyl) - amine has in addition shown interesting activity against certain plant pathogenic fungi.

The substituents R^3, R^4 and R^5 are preferably all hydrogen.

n is preferably 0 or 1.

Where R^3, R^4 and/or R^5 is an aliphatic group this may, for example be a straight chained or branched chained alkyl group, e.g. a methyl, ethyl or butyl group.

The bases according to the invention have a number of basic groups and can therefore form mono-, di- and poly- acid addition salts which are all included within the present invention. The full poly-acid addition salts are preferred: Such salts include, for example, salts with mineral acids, e.g. hydrochlorides, hydrobromides, sulphates, perchlorates, nitrates, phosphates and pyrophosphates, and salts with organic acids, e.g. acetylsalicyclates, formates, acetates, propionates, glycolates, lactates, pyruvates, malonates, succinates, maleates, fumarates, malates, tartrates, citrates, oxalates, benzoates, salicylates, methanesulphonates, ethanesulphonates and *p*-toluene sulphonates.

The compounds according to the invention may be used in human and veterinary medicine in the form of pharmaceutical compositions containing one or more pharmaceutical carriers or excipients suitable, for example, for oral, topical, rectal, intravaginal or parenteral administration. They may be used together with other medicinal agents. The compositions are preferably in dosage unit form and each dosage unit preferably contains 0.5 to 500 mg of the active compound, advantageously 5 to 250 mg, for example 10 to 150 mg.

For administration as solid oral preparations such as tablets or capsules, conventional carriers may be employed, for example, gelatin, lactose, starch, talc, magnesium stearate, hydrogenated oils and polyglycols. The compositions may also take the form of liquid oral preparations for ingestion such as solutions, syrups, elixirs and emulsions, which may contain suspending, emulsifying, stabilising and preserving agents and may also contain acceptable sweetening, flavouring or colouring agents. The compounds may be prepared for local application to the mucous membranes of the nose and throat and may take the form of liquid sprays or powder insufflations, nasal drops, throat paints or similar preparations. Formulations for external applications may be prepared in oily, aqueous or powdered media in the form of conventional skin paints, lotions, creams, ointments, aerosols and dusting powders. Suppositories and pessaries may contain a conventional base, e.g. oil of theobroma, polyglycols and glyco-gelatin bases, together with surface active agents if required. The injectable preparations may take the form of aqueous or oily solutions, emulsions, suspensions or solids for reconstitution before use; Suitable vehicles include, for example, sterile, pyrogen-free water, parenterally acceptable oils, oily esters or other non-aqueous media such as propylene glycol, if desired, containing suspending, dispersing, stabilising, preserving, solubilising, emulsifying or buffering agents.

The pharmaceutical compositions according to the invention preferably contain the active material at a concentration of 0.1 to 95% by weight, advantageously 0.5 to 40%.

For horticultural or agricultural use the compounds according to the invention may be formulated for use in any desired way. Generally such formulations will include the compound in association with a suitable carrier or diluent. Such carriers may be liquid or solid and designed to aid the application of the compound either by way of dispersing it where it is to be applied or to provide a formulation which can be made by the user into a dispersible preparation.

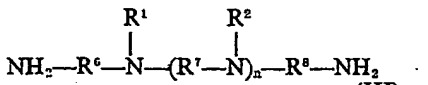
Liquid preparations thus include preparations of the compound in the form of solutions or emulsions which can be used on their own or be adapted to be made up with water or other diluents to form sprays; in such cases the carrier is a solvent or emulsion base non-phytotoxic under the conditions of use. Generally such preparations will include a wetting, dispersing or emulsifying agent. Other liquid preparations include aerosols in which the compound is associated with a liquid carrier or propellant.

Solid preparations include dusts and wettable powders, granulates and pellets, and semi-solid preparations such as pastes. Such preparations may include inert solid or liquid diluents such as clays, which may themselves have wetting properties, and/or wetting, dispersing or emulsifying agents; binding and/or adhesive agents may also be included. Solid

preparations also include thermal fumigating mixtures wherein the compound is associated with a solid pyrotechnic component. In these formulations, the concentration of active material is preferably between 0.01% and 40% *by weight*.

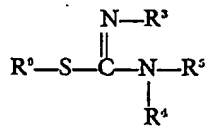
The compounds according to the invention may be prepared in any convenient way, advantageously by the following method.

A compound of the general formula



(III)

may be reacted with a thiourea derivative of the general formula



(IV)

(where R^9 is an alkyl or aralkyl group, e.g. a methyl, ethyl, propyl, butyl or benzyl group, and R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8 and n have the meanings given above, or an acid addition salt thereof, e.g. a mineral acid salt such as a hydrohalide, a nitrate or a sulphate.

The reaction may be effected in the presence or absence of a solvent, suitable solvents, where present, including water and water-miscible organic solvents such as water-miscible alcohols, ethers, ketones, or carboxylic acids, e.g. methanol, ethanol, propanol, dioxan, tetrahydrofuran, acetone, methyl ethyl ketone and acetic acid. The reaction temperature is not especially critical and normal temperature is generally convenient, although higher temperatures, e.g. up to the boiling point of the medium, can also be used.

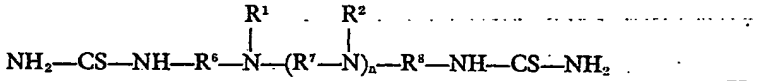
Other useful preparative methods for obtaining the new compounds include:—

1) Reaction of cyanamide with the amine of formula (III) given above, where R^1, R^2, R^6, R^7, R^8 and n have the meanings given above with respect to formula (III), to give compounds having unsubstituted guanidine groups.

2) Reaction of the amine (III) with N-nitroso guanidine; this method also gives an unsubstituted guanidine group. The nitroso-guanidine may, however, carry an N-alkyl substituent to yield an N-alkyl guanidine.

3) Reaction of the amine (III) with a cyanogen halide followed by reaction with ammonia or an amine.

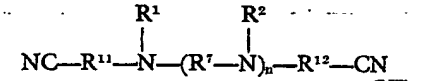
4) Reaction of substituted thiourea of the general formula



(V)

where R^1, R^2, R^6, R^7, R^8 and n have the meanings given above, with mercuric oxide and an amine or ammonia.

The amines of formula (III) used in the methods given above can be conveniently prepared by reduction of the corresponding nitriles of the general formula



(VI)

where R^1, R^2, R^7 and n have the meanings given above with respect to formula (III) and R^{11} and R^{12} are similar to R^6 and R^8 respectively except that they contain one less carbon atom each.

The above nitriles can be prepared, for example, by the addition of an ethylenically unsaturated nitrile such as acrylonitrile to an alkylene diamine or polyalkylene polyamine. The use of acrylonitrile will lead to amines of formula (III) having terminal

$$-NH(CH_2)_3\ NH_2$$

groups.

The reduction of the nitrile of formula (VI) is preferably effected by hydrogenation under pressure at an elevated temperature using a nickel catalyst, in a solvent such as ethanolic ammonia (10%). Pressures of from 90—100 atmospheres and temperatures of about 100° C may conveniently be used.

The amines of formula (III) can also be prepared by condensation of alkylene diamines or polyalkylene polyamines with alkylene dihalides, preferably the chlorides or bromides. It is to be noted that mixed dihalides may be used, for example 1,3-chlorobromopropane. The liberated hydrogen halide is neutralised for example by conversion into the corresponding alkali metal halide, using an alkali metal ethylate in solution in a solvent such as ethanol. The solvent may then be removed, e.g. by distillation, the alkali metal halide extracted and the amine of formula (III) separated by fractional distillation. If the boiling point of the amine is too high for distillation it is sometimes possible to convert the crude amine into the guanidine of formula (I) without further purification.

By analogy with the process given above it is also possible to prepare the amines of formula (III) by condensing amines or

alkylene diamines with cyanhydrins followed by reduction.

Where the preparative method leads to an unsubstituted guanidine group, the substituents R^3, R^4 and R^5 may be introduced subsequently by alkylation, for example using conventional techniques such as reaction with an alkyl halide, sulphate or aromatic-sulphonate alkylating agent.

The methods of preparation of the new compounds described herein will in general give rise to the compounds in the form of acid addition salts. The free base may be prepared from the acid addition salt by treatment with a strong anion exchange resin or treatment with caustic alkali or silver oxide. Other acid addition salts may be formed from the free base so obtained. Alternatively if it is desired to change the anion of an acid addition salt, conventional ion exchange techniques may be used, e.g. using anion exchange resins.

The following Tables I to VII show the results of testing carried out against various pathogens. Table I shows the results of a Tube Dilution Assay against certain human fungi and pathogenic bacteria in respect of the products of Examples 7, 8 and 9.

Table II shows the results of a Tube Dilution Assay against various strains of *Candida albicans*, at various dilutions, in respect of the products of Examples 1, 2, 4 and 5. Table III shows the LD_{95} values for the products of Examples 2, 7 and 8 against certain plant pathogenic fungi as determined by the method of Pianka and Hall (J.Sci.Food and Agric. 1957, 432).

Table IV shows the activity of the products of Examples 7 and 8 against *Botrytis fabae*, *Venturia inaequalis* and *Uromyces fabae* assessed by the leaf disc technique.

Table V shows the results of testing the products of Examples 5, 7, 8 and 9 against *Venturia inaequalis*, *Erysiphe graminis* and *Podosphaera leucostricha* by inoculating both treated and untreated leaves with the pathogen spores (Cross, McWilliam and Rhodes, J.Gen.Microbiol.*34*, 51—65,1964).

Table VI shows the results of growth tests against a number of seed disease organisms in respect of the products of Examples 5 and 7.

Table VII shows results of in vitro minimum inhibitory concentration tests against various pathogens on agar plates (Cup Plate Assays) in respect of the products of Examples 5, 7, 8 and 9.

TABLE I

Compound	Minimum inhibitory concentrations				
	C. Albicans	S. Aureus	E. Coli	Ps. Pyocyaneus	B. Subtilis
Bis-(12-Guanidinododecyl)amine hydrochloride (Example 8)	1—5 μg/ml	1—5 μg/ml	1—5 μg/ml	5—50μg/ml	1—5 μg/ml
Bis-(10-Guanidinodecyl)amine sulphate (Example 7)	1—5 μg/ml	1.5 μg/ml	5—50μg/ml	5—50μg/ml	1—5 μg/ml
Bis-(8-Guanidinooctyl)amine sulphate (Example 9)	1—5 μg/ml	5.50 μg/ml	5—50μg/ml	100 μg/ml	5—50μg/ml

TABLE II

Strain of Candida albicans	Inoculum level (orgs/ml)	Minimum inhibitory concentration (μg/ml)			
		Product of Example 1	Product of Example 2	Product of Example 4	Product of Example 5
C 316	$5{,}000 \times 10^6$	>250	>250	>250	250
	50×10^6	<31.2	<31.2	<31.2	<31.2
	5×10^5	<31.2	<31.2	<31.2	<31.2
Stockton	$5{,}000 \times 10^6$	>250	>250	>250	62.5
	50×10^6	<31.2	<31.2	<31.2	<31.2
	5×10^5	<31.2	<31.2	<31.2	<31.2
Woods	$5{,}000 \times 10^6$	>250	>250	>250	250
	50×10^6	125	125	<31.2	<31.2
	5×10^5	<31.2	<31.2	<31.2	<31.2
Gregory	$5{,}000 \times 10^6$	>250	>250	>250	250
	50×10^6	<31.2	<31.2	62.5	<31.2
	5×10^5	<31.2	<31.2	<31.2	<31.2

TABLE III

Compound	LD_{95}			
	Cercospora melonis	Venturia inaequalis	Botrytis cinerea	Fusarum bulbigenum
N,N'-bis-(3-guanidinopropyl)hexamethylenediamine hydrochloride	>100	90	>10	>100
bis-(10-guanidinodecyl)-amine sulphate	>10	18	>10	>50
bis-(12-guanidinododecyl)-amine hydrochloride	>10	25	>10	>50

TABLE IV

Compound	Conc.	Botrytis fabae % Kill	Uromyces fabae % Kill	Venturia inaequalis
bis-(10-guanidinodecyl)-amine sulphate	100	97	99+	—
	50	98	—	—
	10	91	—	—
bis-(12-guanidinododecyl)-amine hydrochloride	300	—	—	96
	100	97	97	—
	50	94	—	—
	10	46	—	—

TABLE V

Product of	*Venturia inaequalis* (*apple scab*) *spore germination test* Minimum inhibiting concentration in p.p.m.	*Apple scab greenhouse test* Compounds sprayed on leaves of potted apple rootstocks % *reduction of disease when compared with untreated leaves*		Barley mildew (*Erysiphe graminis*) *greenhouse test* Compounds sprayed on leaves of barley plants in pots % *reduction of disease when compared with untreated leaves*	*Apple mildew* (*Podosphaera leucotricha*) *greenhouse test* Compounds sprayed on leaves of potted apple rootstocks % *reduction of disease when compared with untreated leaves*	
		200 ppm	50 ppm	200 ppm	200 ppm	100 ppm
Example 5	10			19%		
Example 7	10	83%	57%	76%	39%	40%
Example 8	5	41%		5%	18%	17%
Example 9	20	99%	94%	98%	77%	74%

TABLE VI

Product of	Growth Tests against selected seed disease organisms: Figures represent percentage of healthy plants in respectively, the treated and the untreated batches of plants										Figures represent number of diseased roots in respectively, the treated and untreated plants	
	Fusarium culmorum		Fusarium graminearum		Fusarium nivale		Phoma betae		Xanthomonas malvacearum		Ophiobolus graminis	
Example 5			36%	32%		43%		21%				
Example 7	95%	68%	47%	32%	54%	10%	45%	21%	53%	38%	24	40

TABLE VII

In vitro laboratory tests on agar plates

Zone sizes given by the compounds when assayed against the named seed disease organisms

Concentrations in ppm Zone sizes in mm

Product of	Ustilago nuda		Ustilago hordei		Ustilago kolleri		Ustilago avenae		Helmintho-sporium gramineum	
	200ppm	20ppm	200	20	200	20	200	20	200	20
Example 5	15.8 mm	0							Trace	0
Example 7	12.2 mm	0	13.4 mm	0	17.0	0	14.8	0	21.0	14.6
Example 8			Trace	0	Trace	0	Trace	0	0	0
Example 9	0	0	18.6	17.6	25.7	21.8	31.5	31.0	18.9	12.3

Product of	Helmino-hosporium avenae		Fusarium culmorum		Fusarium nivale		Phome betae		Xanthomonas medicaginis	
	200ppm	20ppm	200	20	200	20	200	20	200	20
Example 5	18.2	0	21.5	0	14.2	0	15.1	11.2		
Example 7	18.1	13.6	18.3	13.7	16.8	11.8	18.0	13.6	19.0	0
Example 8	12.5	0	0	0	11.4	Trace	11.1	0	15.5	0
Example 9	19.6	15.5	28.2	28.7	28.0	24.3	16.1	Trace	0	0

Product of	Ophiobolus graminis		Pythium aphenid-ermatum		Pseudomonas medicaginis		Ustilago mayolis		Rhizoctona solani	
	200ppm	20ppm	200	20	200	20	200	20	200	20
Example 5	0	0	18.2	14.2	0	0			0	0
Example 7	24.9	15.0	19.9	0	0	0	18.6	11.0	21.8	15.0
Example 8	0	0	Trace	0	11.5	0	12.5	0	13.8	0
Example 9	24.9	26.0	31.9	31.0	0	0			0	0

In order that the invention may be well understood we give the following Examples by way of illustration only (all temperatures are in °C):—

EXAMPLE 1

Acrylonitrile (97.1 grams) was added slowly with stirring to bis-(3-aminopropyl)amine (100.4 grams), the temperature being kept below 30° C. The mixture, after standing for 3 days, was hydrogenated at 100° C and 100 atmospheres pressure in the presence of ethanolic ammonia solution (200 millilitres of 10%) and Nicat NP—AC60 nickel catalyst (registered Trade Mark) (5 grams). After removal of the catalyst by filtration, and ammonia and ethanol by distillation, the residue was fractionated at reduced pressure, giving 21.8 grams of the tetrakis(trimethylene)pentamine of boiling point 125—130°/0.07 mm. Hg.

A solution of 83.7 grams of S-methylisothiouronium sulphate in 270 millilitres of water was well stirred with 72.5 grams of tetrakis - (trimethylene)pentamine, and the mixture allowed to stand at room temperature for 24 hours. After heating for 1 hour to drive off the methyl mercaptan which was formed, 300 millilitres of 3N-sulphuric acid were added and sufficient ethanol to allow crystallisation to take place. The solid which formed was collected and recrystallised from a mixture of water and ethanol. Bis-[3-(3-guanidinopropylamino)propyl]amine sulphate

$$[NH_2.C.(=NH).NH.(CH_2)_3.NH.(CH_2)_3.NH.(CH_2)_3.NH.(CH_2)_3.NH.C.(=NH).NH_2.]_2.5H_2SO_4,$$

was a white solid of melting point 265—268° C.

EXAMPLE 2

Acrylonitrile (150 millilitres) was slowly added with stirring to a solution of hexamethylenediamine (116 grams) in 120 millilitres of water, the temperature being maintained below 30° C. After the addition was completed, the mixture was stirred for a further half hour and then allowed to stand overnight. The excess of acrylonitrile and the water were removed by distillation under reduced pressure on the steam bath. The residue was dissolved in ethanolic ammonia (10%) and hydrogenated at 100° C and 90—100 atmospheres pressure in the presence of 100 grams of Nicat NP AC—60 nickel catalyst. After removal of the catalyst, ammonia and alcohol, the residue was fractionally distilled yielding N-(3-aminopropyl)hexamethylenediamine (49.8 grams b.p. 166—181° C) in the fore-run, and N,N'-bis - (3 - aminopropyl)hexamethylenediamine (37.9 grams b.p. 167—169°/1.2 mm. Hg.).

A mixture of 11.6 grams of N,N'-bis-(3-aminopropyl)hexamethylenediamine and 13.9 grams of S-methylisothiouronium hydrochloride in 150 millilitres of water was allowed to stand at room temperature overnight and then heated for one hour on a steam bath. The water was removed by distillation under reduced pressure and the gummy residue was solidified by treatment with acetone. The product which weighed 21.7 grams was twice recrystallised from ethanol/water mixture to give N,N'-bis - (3 - guanidinopropyl)hexamethylenediamine hydrochloride of formula:—

$$NH_2.C.(=NH).NH.(CH_2)_3.NH.(CH_2)_6.NH.(CH_2)_3.NH.C(=NH).NH_2.4HCl.$$

of m.p. 279° C.

EXAMPLE 3

N,N' - bis - (3 - aminopropyl)hexamethylenediamine was prepared using the procedure of the first part of Example 2.

S - Methylisothiouronium sulphate (15.3 g.) was dissolved in water (100 millilitres) and converted to the corresponding hydrochloride using barium chloride; the volume of the solution was then adjusted to 150 millilitres. This solution was then added to 11.6 g. of N,N' - bis - (3 - aminopropyl)-hexamethylenediamine, swirled until mixed and then allowed to stand overnight. After heating for a further hour on a steam bath, 2N hydrochloric acid (50 millilitres) was added, the water removed by vacuum distillation over a steam bath and the residue hardened with acetone. 21.7 g. of N,N'-bis - (3 - guanidinopropyl)hexamethylenediamine hydrochloride having a melting point of 272° C were obtained. Yield 94.4%. On recrystallisation from ethanol/water the melting point was 279° C.

EXAMPLE 4

1,3 - Bromochloropropane (240 grams) was rapidly added to a solution of hexamethylenediamine (1044 grams) in ethanol (800 millilitres) contained in a flask provided with a reflux condenser. The heat of reaction caused the mixture to boil and boiling was maintained by external heating for 18 hours. After cooling, a solution of sodium (69 grams) in ethanol (1 litre) was slowly added with stirring and the precipitated salts afterwards removed by filtration. The alcohol was distilled off under a vacuum and the residue stirred with benzene (2 litres). A further lot of precipitate salts were removed by filtration. The benzene was distilled off and the residue fractionally distilled. There were thus ob-

tained 627 grams of unreacted hexamethylenediamine and 324 grams (61%) of N,N'-bis - (6 - aminohexyl)trimethylenediamine of b.p. 176°/0.2 mm. Hg.

A mixture of 12.5 grams of S-methylisothiouronium sulphate and 13.6 grams of N,N'-bis - (6 - aminohexyl)trimethylenediamine in 60 millilitres of water was allowed to stand for 16 hours at room temperature and then heated for 1 hour on a steam bath. On cooling and addition of 33 millilitres of 3N-sulphuric acid, a crop of crystals weighing 20.6 grams was deposited which after recrystallisation from water gave pure N,N'-bis-(6-guanidinohexyl)trimethylenediamine sulphate of m.p. 219° C.

$$NH_2.C.(=NH).NH.(CH_2)_6.NH.(CH_2)_3.NH.(CH_2)_6.NH.C(=NH).NH_2.2H_2SO_4.$$

EXAMPLE 5

A solution of 1,6-dibromohexane (27.5 grams) and hexamethylenediamine (78 grams) in ethanol (150 millilitres) was heated under reflux for 18 hours. A solution of sodium (5.3 grams) in ethanol (100 millilitres) was added and the alcohol then removed by distilling off under reduced pressure. The residue was extracted with four 250 millilitre quantities of ether. The ether from the combined extracts was removed by evaporation and the residue on fractionation furnished 57 grams of unchanged hexamethylenediamine and 21.3 grams of tris-hexamethylenetetramine which was used without further purification.

A mixture of tris-(hexamethylene)tetramine (21.3 grams) and S-methylisothiouronium sulphate (16.4 grams) in water (120 millilitres) was allowed to stand overnight at room temperature and the evolution of methyl mercaptan which took place was completed by heating for 2 hours on a steam bath. Addition of 46 millilitres of 3N-sulphuric acid followed by excess of ethanol caused precipitation of a white solid which was recrystallised from water to give N,N' - bis - (6-guanidinohexyl)hexamethylenediamine sulphate,

$$NH_2.C.(=NH).NH.(CH_2)_6.NH.(CH_2)_6.NH.(CH_2)_6.NH.C(=NH).NH_2.2H_2SO_4$$

of m.p. 256° C.

EXAMPLE 6

A mixture of bis-(6-aminohexyl)amine (10.8 grams) and S-methylisothiouronium sulphate (15 grams) in water (150 millilitres) was allowed to react for 16 hours at room temperature followed by one hour's heating on a steam bath. Sulphuric acid (16.7 millilitres of 3N-) were added and the heating continued for 1 hour. The water was removed by distillation at reduced pressure and the residue was twice recrystallised from aqueous alcohol. There were thus obtained 12.3 grams of bis-(6-guanidinohexyl)amine sulphate

$$[NH_2.C.(=NH).NH.(CH_2)_6.NH.(CH_2)_6.NH.C(=NH).NH_2]_2.3H_2SO_4.$$

of m.p. 247°.

EXAMPLE 7

To a solution of 3 grams of S-methylisothiouronium sulphate in 15 millilitres of water were added 3.3 grams of bis-(10-aminodecyl)amine. The mixture was heated for one hour on a boiling water bath whilst methyl mercaptan was eliminated. After addition of 3.3 ml. of 3N-sulphuric acid and cooling, the white solid which separated, was filtered off and twice recrystallised from water yielding 3.15 grams of bis-(10-guanidinodecyl)amine sulphate

$$NH_2.C.(=NH).NH.(CH_2)_{10}.NH.(CH_2)_{10}.NH.(=NH).C.NH_2.1.5H_2SO_4$$

of m.p. 207°C.

The bis - (10 - aminodecyl)amine was obtained from the higher boiling fractions following the catalytic hydrogenation of sebaconitrile using a nickel catalyst, and had b.p. 218—220°/0.7 mm. Hg.

EXAMPLE 8

To a solution of S-methylisothiouronium hydrochloride, prepared by treating 23.7 grams of S-methylisothiuronium sulphate with 20.8 grams of barium chloride dihydrate in 300 millilites of water and filtering off the precipitated barium sulphate, were added 30 grams of bis-(12-aminododecyl)amine. The mixture was heated for three hours on a steam bath, treated with 39.5 millilitres of 3N-hydrochloric acid, and then concentrated to a gummy residue by evaporation at reduced pressure. The residue was hardened by treatment with acetone and weighed 32.4 grams. Following recrystallisation from a mixture of equal parts by volume of glacial acetic acid and acetone, the so obtained bis-(12-guanidinododecyl)amine hydrochloride

$NH_2.C.(=NH).NH.(CH_2)_{12}.NH.(CH_2)_{12}.NH.(=NH).C.NH_2.3HCl$,

had m.p. 195° C.

EXAMPLE 9

Bis - (8 - guanidinooctyl)amine sulphate

$NH_2.C.(=NH).NH.(CH_2)_8.NH.(CH_2)_8.NH.(=NH).C.NH_2.1.5H_2SO_4$

of m.p. 234° C was prepared in a manner similar to that described in Example 7, from bis - (8 - aminooctyl)amine (18.2 grams), and S-methylisothiouronium sulphate (20.2 grams), in water (380 millilitres). The final solution was treated with 22.2 millilitres of 3N-sulphuric acid, concentrated *in vacuo* and the residue recrystallised from aqueous isopropanol.

EXAMPLE 10

A mixture of 20.3 grams of N,N'-bis(6 - aminohexyl)isopropylene diamine b.p. 184—7°/0.35 mm. Hg, (prepared by the reaction of 1,6-diaminohexane with 1,2-dichloropropane in ethanol), 22.1 grams of S-methylisothiouronium sulphate and 130 millilitres of water was allowed to stand at room temperature overnight. After heating for two hours on a steam bath, the solution was treated with 49 millilites of 3N-sulphuric acid. The gummy residue which resulted after concentrating the liquor *in vacuo*, was extracted with ethanol, and the insoluble portion recrystallised from 50% aqueous methanol. The so obtained N,N' - bis - (6 - guanidinohexyl)isopropylenediamine sulphate

$NH_2.C.(=NH).NH.(CH_2)_6.NH.CH.(CH_3).CH_2.NH.(CH_2)_6.NH.C.(=NH).NH_2.2H_2SO_4$.

had m.p. 303—304° C.

In the following examples mesh sizes referred to are British Standard meshes.

EXAMPLE 11

Oral Elixir

Formula:

N:N'-Bis-(6-guanidinohexyl) trimethylenediamine sulphate (Example 4)		1 g.
Ethanol 60 O.P.		10 ml.
Sucrose B.P.		30 g.
Methyl hydroxybenzoate B.P.		150 mg.
Propyl hydroxybenzoate B.P.		100 mg.
Flavouring and colouring agents		q.s.
Water	to	1000ml.

Method of preparation:

1. Dissolve sucrose in 50 ml. water, add N:N'-bis-(6-guanidinohexyl) trimethylenediamine sulphate and dissolve.
2. Dissolve methyl and propyl hydroxybenzoates in alcohol, add flavouring agents, mix and add to the aqueous solution.
3. Add a solution of the colouring agents; make up to volume with water, mix well and filter.

Dose:

5 ml. (one teaspoonful) of the elixir contains 50 mg. of N:N'-bis-(6-guanidinohexyl) trimethylenediamine sulphate.

EXAMPLE 12

Injection 10 mg. per ml.

Formula:

N:N'-Bis-(6-guanidinohexyl)hexamethylenediamine sulphate (Example 5)		1.0 g.
Water for injection B.P.	to	100 ml.

Method of preparation:

Dissolve N:N'-Bis-(6-guanidinohexyl)hexamethylenediamine sulphate in sufficient water for injection to make 100 ml. Sterilise by filtration through a 5/3 sintered glass filter. Aseptically pack in sterile 1 ml. ampoules.

EXAMPLE 13

Tablet. 50 mg.

Formula:

N:N'-Bis-(6-guanidinohexyl)trimethylenediamine sulphate (Example 4) in fine powder	50.0 mg.
Lactose B.P.	77.5 mg.
Starch, B.P. maize	22.5 mg.
10% w/v maize starch paste	q.s.
Magnesium stearate	1.0% w/w

Method of preparation:

1. Blend the powders and damp with starch paste. Mix thoroughly.
2. Pass the wet mass through a 12 mesh screen and dry the resultant granules at 50° C.
3. Screen the dry granules 18 mesh and blend with the magnesium stearate.
4. Compress into tablets.

Compression weight:— 0.155 g.

EXAMPLE 14

Capsule, 50 mg.

Formula:

N:N'-Bis-(6-guanidinohexyl)trimethylenediamine sulphate (Example 4) in fine powder	50 mg.
Lactose B.P. in fine powder	200 mg.

Method of preparation:

1. Blend the powders.
2. Fill into No. 2 hard gelatin capsules filling 250 mgm. into each capsule.

EXAMPLE 15

Dusting Powder 5% w/w

Formula:

Bis-(10-guanidinodecyl)amine sulphate (Example 7) in fine powder		5 g.
Starch		10 g.
Purified Talc, sterilised	to	100 g.

Method of preparation:

1. Sift the powders through a 100 mesh screen.
2. Blend.

EXAMPLE 16

Cream 5% w/w

Formula:

Bis-(12-guanidinododecyl)amine hydrochloride (Example 8)	50 g.
Emulsifying wax B.P.	90 g.
White soft paraffin	150 g.
Liquid paraffin	60 g.
Chlorocresol	1 g.
Purified Water	649 g.

Method of preparation:

1. Dissolve the bis-(12-guanidinododecyl) amine hydrochloride and chlorocresol in the purified water with the aid of gentle heat, if necessary.
2. Melt the other ingredients and add the aqueous solution gradually with stirring.
3. Stir until cold and homogenise.

Example 17

Ointment 1% w/w

Formula:

Bis-(12-guanidinododecyl) amine hydrochloride (Example 8) in fine powder	10 g.
Wool fat	90 g.
White soft paraffin	900 g.

Method of preparation:

1. Melt the wool fat and white soft paraffin. Stir until cold.
2. Incorporate the bis-(12-guanidinododecyl) amine hydrochloride mixing intimately.

EXAMPLE 18

Wettable powder 25%

Formula:

Bis-(12-guanidinododecyl)-amine hydrochloride		25 parts
Fatty alcohol sulphonate		0.5 parts
Calcium lignosulphonate		6 parts
China clay	to	100 parts

EXAMPLE 19

Solution 0.05%

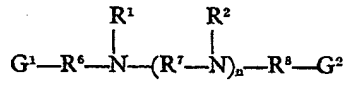
Formula:

Bis-(12-guanidinodecyl)-amine hydrochloride	5 parts
Water	10,000 parts

In Examples 18 and 19 the active substance can, if desired, be replaced by an equivalent quantity bis - (10 - guanidinodecyl) - amine hydrochloride or bis - (8-guanidinooctyl) - amine sulphate.

WHAT WE CLAIM IS:—

1. Compounds of the general formula



(I)

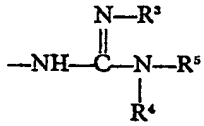
and their physiologically acceptable acid addition salts, where

R^1 and each radical R^2, which may be the same or different, are hydrogen atoms or alkyl groups,

R^6 and each group R^7 and R^8, which may be the same or different, are straight or branched alkylene groups separating the adjacent nitrogen atoms by chains of at least two carbon atoms, the total number of carbon and nitrogen atoms in the straight chain linking the substituents G^1 and G^2, excluding branching groups, being greater than 12,

n is an integer from 0 to 4, and

G^1 and G^2, which may be the same or different, have the formula



(II)

where R^3, R^4 and R^5, which may be the same or different, are hydrogen atoms or alkyl groups having 1—4 carbon atoms.

2. Compounds as claimed in claim 1 in which one or more of the chains R^6, R^7 and R^8 contained 6 or more carbon atoms.

3. Compounds as claimed in claim 1 or claim 2 in which the chains R^6, R^7 and R^8 each contain not more than 20 carbon atoms.

4. Compounds as claimed in claim 1 in which the chains R^6, R^7 and R^8 each contain not more than 15 carbon atoms.

5. Compounds as claimed in any of the preceding claims in which the chains R^6, R^7 and R^8 each contain 6—12 carbon atoms.

6. Compounds as claimed in any of the preceding claims in which R^3, R^4 and R^5 are all hydrogen.

7. Compounds as claimed in any of the previous claims in which n is 0 or 1.

8. N,N′ - bis - (3 - guanidinopropyl)-hexamethylene diamine.

9. N,N′ - bis - (6 - guanidinohexyl) - hexamethylene diamine.

10. N,N′ - bis - (6 - guanidinohexyl) - trimethylene diamine.

11. Bis - (8 - guanidinooctyl) - amine.

12. Bis - (10 - guanidinodecyl) - amine.

13. Bis - (12 - guanidinododecyl) - amine.

14. Bis - [3 - (3 - guanidinopropylamino)-propyl] - amine.

15. Bis - (6 - guanidinohexyl) - amine.

16. N,N′ - Bis - (6 - guanidinohexyl) - isopropylenediamine.

17. The hydrochlorides, hydrobromides, sulphates, perchlorates, nitrates, phosphates,

pyrophosphates, acetylsalicylates, formates, acetates, propionates, glycolates, lactates, pyruvates, malonates, succinates, maleates, fumarates, malates, tartrates, citrates, oxalates, benzoates, salicylates, methanesulphonates, ethanesulphonates, and *p*-toluene sulphonates of the bases as claimed in any of the preceding claims.

18. Compounds as claimed in claim 1 as herein disclosed.

19. Pharmaceutical compositions comprising one or more compounds as claimed in claim 1 as active material together with a pharmaceutical carrier or excipient.

20. Compositions as claimed in claim 19 in forms suitable for oral, topical, rectal, vaginal or parenteral administration.

21. Compositions as claimed in claim 19 or claim 20 in dosage unit forms.

22. Compositions as claimed in claim 21 in which each dosage unit contains 0.5 to 500 mg. of active compound.

23. Compositions as claimed in claim 21 in which each dosage unit contains 5 to 250 mg. of active compound.

24. Compositions as claimed in claim 21 in which each dosage unit contains 10 to 150 mg. of active compound.

25. Compositions as claimed in any of claims 19 to 24 which contain 0.1 to 95% by weight of active material.

26. Compositions as claimed in claim 25 which contain 0.5 to 40% by weight of active material.

27. Compositions as claimed in any of claims 19 to 26 in the form of tablets, capsules, solutions, syrups, elixirs, emulsions, sprays, powder insufflations, nasal drops, throat paints, skin paints, lotions, creams, ointments, aerosols, dusting powders, suppositories, pessaries or injectable preparations.

28. Pharmaceutical compositions as claimed in claim 19 substantially as herein described.

29. Pharmaceutical compositions as claimed in claim 19 substantially as herein described with reference to any of Examples 11—17.

30. Compositions for use in horticulture and/or agriculture comprising one or more compounds as claimed in claim 1 together with a diluent or extender.

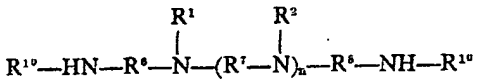
31. Compositions as claimed in claim 30 in the form of solutions or wettable powders.

32. Compositions as claimed in claim 30 or claim 31 containing 0.01 to 40% by weight of active material.

33. Compositions as claimed in claim 30 substantially as herein described.

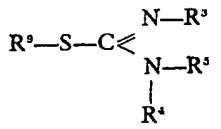
34. Compositions as claimed in claim 30 substantially as herein described with reference to Example 18 or Example 19.

35. A process for the preparation of compounds as claimed in claim 1 in which an amine of the general formula



(III)

is reacted, where R^{10} is hydrogen, with a thiourea derivative of the general formula



(IV)

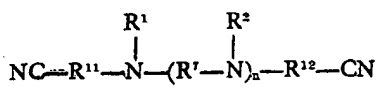
(where R^9 is an alkyl or aralkyl group, R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8 and n have the meanings given in claim 1), or an acid addition salt thereof, or with cyanamide, with N - nitrosoguanidine, with an N - alkyl-N - nitrosoguanidine or with cyanogen halide followed by reaction of the cyanogen-halide reaction-product with ammonia or an amine or is reacted, where R^{10} is a group $CS.NH_2$, with mercuric oxide and ammonia or an amine.

36. A process as claimed in claim 35 in which the amine of formula III is reacted with the thiourea derivative in the presence of water or a water-miscible organic solvent.

37. A process as claimed in claim 36 in which the solvent is a water-miscible alcohol, ether, ketone or carboxylic acid.

38. A process as claimed in any of claims 35 to 37 in which R^9 in the thiourea derivative of formula IV is a methyl, ethyl, propyl, butyl or benzyl group.

39. A process as claimed in any of claims 35 to 38 in which the amine of formula III is prepared by reduction of a nitrile of the general formula



(V)

where R^1, R^2, R^7 and n have the meanings given in claim 1 and R^{11} and R^{12} are alkylene chains having 1 carbon atom less than R^6 and R^8 respectively, R^6 and R^8 having the meanings given in claim 1.

40. A process as claimed in claim 39 in which the reduction of the nitrile is preferably effected by hydrogenation using a nickel catalyst.

41. A process as claimed in claim 40 in which hydrogenation is effected under pressure at elevated temperature in ethanolic ammonia.

42. A process as claimed in any of claims 39 to 41 in which the nitrile of formula V is prepared by condensing an ethylenically unsaturated aliphatic nitrile with an alkylene diamine or polyalkylene polyamine.

43. A process as claimed in any of claims 35 to 38 in which the amine of formula III is prepared by condensing an alkylene diamine or polyalkylene polyamine with an alkylene dihalide.

44. A process as claimed in any of claims 35 to 38 in which the amine of formula III is prepared by reacting an amine or alkylene diamine with a cyanhydrin followed by reduction.

45. A process as claimed in any of claims 35 to 44 in which a free base of formula I initially produced is converted into an acid addition salt thereof.

46. A process as claimed in any of claims 35 to 44 in which where R^3, R^4 and/or R^5 in the product of formula I is hydrogen, the product is reacted with an alkylating agent.

47. A process as claimed in claim 35 substantially as herein described.

48. Compounds as claimed in claim 35 substantially as herein described with reference to any of Examples 1—10.

49. Compounds as claimed in claim 1 whenever prepared by a process as claimed in any of claims 35 to 47.

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